CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

ROTENONE

Chemical Code # 518, Tolerance # 50476
SB 950 # 284

October 29, 19887
Revised 12/22/87, 9/9/94, 4/8/96, 12/24/96, 2/18/97

I. DATA GAP STATUS (See footnote)

Chronic toxicity, rat:	Data gap, inadequate studies, no adverse effect indicated
Chronic toxicity, dog:	Data gap, inadequate studies, no adverse effect indicated
Oncogenicity, rat:	Data gap, inadequate studies, possible adverse effect indicated
Oncogenicity, mouse:	Data gap, inadequate studies, no adverse effect indicated
Reproduction, rat:	Data gap, inadequate studies, possible adverse effect indicated
Teratology, rat:	Data gap, inadequate studies, possible adverse effect indicated
Teratology, mouse:	Data gap, inadequate studies, possible adverse effect indicated
Gene mutation:	Data gap, inadequate studies, possible adverse effect indicated
Chromosome effects:	Data gap, inadequate studies, possible adverse effect indicated
DNA damage:	Data gap, inadequate studies, possible adverse effect indicated
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 151307 and 915999 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T961224
Revised by Stanton Morris, 12/24/96; J. Gee, 2/18/97

The major deficiency in the long term studies has been identified as the lack of ophthalmology. This remains true. However, a number of studies in the rat, mouse and dog have included clinical observations, macroscopic findings and histological evaluation of the eyes. It is, therefore, unlikely that any significant adverse effect on the eye would have been missed. While no one study would be sufficient to address ophthalmology, the collective data from a number of studies, including those in the open literature, plus the known mechanism of action, can be used to satisfy the requirement for examination of the eye. In addition, documents from the US EPA do not identify ophthalmology as a

concern. The lack of hematology in the oncogenicity studies is not considered a major deficiency. The rat reproduction study and the rat teratology have no deficiency identified other that the test material. The mouse teratology study, when considered with the range-finding study, provides adequate data. Although no one mutagenicity study is acceptable, collectively the data indicate that rotenone is not mutagenic in prokaryotes but suggest that is mutagenic in some mammalian systems. The evidence for carcinogenicity is mixed in that some studies are negative and others are positive. The study conducted for the National Toxicology Program (NTP) was negative in the mouse but suggestive of a positive effect in male rats for adenomas in the parathyroid. The Peer Review Panel of that study, however, were not unanimous in their interpretation of the pathology in the male rat. At this time, there does not appear to be a need for replacement studies in any of the areas defined by SB950 of 1984.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

CHRONIC TOXICITY, RAT

50476-027; 095175; "Chronic Toxicity of Rotenone in Rats", Study No. 6115-100; M. Tisdel; Hazleton Laboratories America, Inc., Madison, WI; 12/30/85. Recrystallized rotenone (lot no. 215-LCD-1 L19123, 96.4% analytical purity) was fed in the diet of 40 Fischer 344 rats/sex/group for 24 months at 0, 7.5, 37.5, or 75 ppm. Treatment-related effects at 37.5 and 75 ppm were lower body weight gains for both sexes and decreased food consumption for females (NOEL = 7.5 ppm). There were sporadic treatment-related effects on serum levels of total protein, albumin, and urea nitrogen. No adverse effect was indicated. The study was unacceptable and not upgradeable because the technical active ingredient was not used and there were no ophthalmological data (J. Kishiyama and S. Morris, 4/8/94; S. Morris and J. Gee, 4/8/96).

> 50476-059; 150209: EPA' s evaluation of DPR doc. # 50476-027, rec. # 095175 - Supplementary.
>
> 50476-051; 145631: The registrant submitted comments on DPR's evaluations, a rationale for not using the registered technical active ingredient, the study protocol, and data on ocular lesions seen in rats exposed for two years to dietary mixtures of rotenone. Evaluation of this submission resulted in a change in study status to not upgradeable. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-020; 063487; "Studies on the Chronic Toxicity of Pro-Noxfish, a Proprietary Synergized Rotenone Fish-Toxicant." (S. B. Penick, published in Toxicol. Appl. Pharmacol. 3: 49-56 (1961), I. C. Brooks and R. W. Price) Pro-Noxfish (rotenone plus sulfoxide); given in drinking water to rats of the Carworth strain at 0 or 100 ppm Pro-Noxfish (2.5% rotenone (100%)); 70 week study with 20 animals per group (both sexes); body weight gain was decreased in the treated males and females; water consumption was also decreased in the 100 ppm group; histopathology on 5/sex in treated group and 3/sex in controls; report states there were negative findings; UNACCEPTABLE (summary). No adverse chronic effects reported. (J. Gee, 12/4/87).

50476-020; 064185; "Chronic Toxicity of Cube." (Published in Toxicol. Appl. Pharmacol. 7: 535 - 542 (1965), Hansen, W. H. et al.) Cube powder, 5.80% rotenone, 82.2% inerts and 12% "other extractives"; fed in the diet for two years to Osborne-Mendel rats at 0, 50, 100, 250, 500 or 1000 ppm cube, 50 per group; hematology at 3-, 11-, 17- and 22-months for 5/sex/group; histopathology of tissues from 6/sex at 1000 ppm and 6 males and 5 females in control group; significant decrease in weight gain in females at 100 ppm and above and in males at 500 and 1000 ppm by week 12; no treatment-related effects were noted in hematology, gross or histopathology; nominal NOEL = 50 ppm (decreased weight gain); UNACCEPTABLE (summary). No adverse effect reported other than growth retardation. (J. Gee, 12/4/87).

> 50476-025; 064185: This document contains an exact duplicate of the study at DPR doc. # 50476-020, rec. # 064185.
>
> 50476-002; 032707: This document is a duplicate of the study at DPR doc. # 50476-020, rec. # 064185.
>
> 50476-002; 915999: This document is a duplicate of the study at DPR doc. # 50476-020, rec.

064185.

50476-023; 064228; "Chemicals in Foods: A Report to the Association of Food and Drug Officials on Current Developments. Part II. Pesticides. Section III. Subacute and Chronic Toxicity." (Published in Association of Food & Drug Officials of the United States 16: 47 - 53 (1952), Lehman.) Data in table form only with bases for NOEL's not given. Rats (number not stated) were exposed for 104 weeks to rotenone at apparent doses of 2, 5, 25 or 50 ppm. "Tissue damage" was reported at 5 ppm, gross effects at 25 ppm. UNACCEPTABLE (summary only). (J. Gee, 12/7/87).

CHRONIC TOXICITY, DOG

50476-025; 064238; "Subchronic Oral Dosing Study for Safety Evaluation of Rotenone Using Dogs", MRI Project No. 4853-B; H.V. Ellis, S. Unwin, J. Cox, I.S. Elwood, E.A. Castillo, E.R. Ellis and J. Carter; Midwest Research Institute Report, La Crosse, WI; December 1980. Rotenone (analytical purity > 99%) was given orally in gelatin capsules to 6 beagle dogs/sex/dose at 0, 0.4, 2, or 10 mg/kg/day for 26 weeks. Treatment-related effects were: decreased group mean body weights for females at 2 mg/kg/day and both sexes at 10 mg/kg/day; decreased food consumption at 10 mg/kg/day for both sexes; increased emesis in males at 2 and 10 mg/kg/day; increased abnormal feces in males at 2 mg/kg/day and both sexes at 10 mg/kg/day; and both sexes had altered hematology and serum chemistry values at 10 mg/kg/day (NOEL = 0.4 mg/kg/day). No adverse effect was indicated. The study was unacceptable and not upgradeable because the registered active ingredient was not used and there was no ophthalmoscopic data (J. Gee, 12/16/87; J. Kishiyama and S. Morris, 9/9/94; S. Morris and J. Gee, 4/8/96).

50476-059; 150210: EPA' s evaluation of DPR doc. # 50476-025, rec. # 064238 - Guideline.

50476-029; 092533: This document contains additional data for the study at DPR doc. # 50476-025 rec. # 064238. A supplemental worksheet was done (J. Kishiyama and S. Morris, 9/9/94).

50476-038; 112457: This document contains adequate hematology, urinalysis, body weight and clinical observation data but no ophthalmology data. This information was evaluated in the supplemental worksheet for DPR doc. # 50476-029, doc. # 092533 and DPR response dated 9/9/94 (S. Morris, 9/9/94).

50476-051: The registrant submitted comments on DPR's evaluations, a rationale for not using the registered technical active ingredient, and no data. Evaluation of this submission did not change the "not upgradeable" status of the study. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-002; 032706; "Chronic Toxicity of Cube - Beagle Dogs." (Food and Drug Administration, Toxicol. Appl. Pharmacol. 7: 535-542, 1965) Rotenone, 5.8%, fed to beagles, 2/sex/group at 0, 50, 150 or 400 ppm for 28 months. No adverse effects indicated. UNACCEPTABLE, not upgradeable. No individual data, too few animals, no analysis of dose. (J. Gee, 9-1-85).

50476-002; 915999: This is the same document as that at DPR doc. # 50476-002, rec. # 032706.

50476-020; 064185; "Chronic Toxicity of Cube." (Published in Toxicol. Appl. Pharmacol. 7: 535 - 542 (1965), Hansen, W. H. et al.) Cube powder, 5.80% rotenone, 82.2% inerts and 12% "other extractives";

fed in the diet for 28 months to beagle dogs at 0, 50, 150 and 400 ppm cube, 2/sex/group; hematology at pretest, 2 weeks, 1-, 3- and 6-months, 1 and 2 years; no effects from cube treatment were reported. NOEL not established. UNACCEPTABLE (summary). (J. Gee, 12/4/87).

50476-025; 064239; "Toxicological Studies of Derris elliptica and Its Constituents. I. Rotenone." (Published in J. Pharm. Exper. Therap. 43: 193 - 208 (1931), H. Haag.) Rotenone, no purity stated, given by capsule at 10 mg daily for an unspecified time to each of 5 dogs caused "frequent" emesis; 5 mg daily seldom produced emesis over the one month treatment period; fatty changes in the liver and kidney were noted; NOEL not clear; UNACCEPTABLE (summary report). Publication contains brief references to other studies as well. (J. Gee, 12/16/87).

ONCOGENICITY, RAT

50476-028; 088943; "Toxicology and Carcinogenicity Studies of Rotenone in F344/N Rats and B63F1 Mice", NIH Pub. No. 88-2576; K. M. Abdo; National Toxicology Program, Battelle Columbus Laboratory; January 1988. Rotenone (lot no. 735-RAP-1502) was given in the feed to 50 F344/N rats/sex/dose for 103 weeks at 0, 38, or 75 ppm. Female body weights were decreased at 75 ppm relative to controls but they were always > 90% of controls (non-oncogenic NOEL = 38 ppm). A **possible adverse effect** was indicated by an incidence of parathyroid adenomas in males at 75 ppm. The study was unacceptable and not upgradeable because the registered technical active ingredient was not used, the rationale for the doses was not adequate, and there were no hematology data and no histopathology data for the spinal cord (J. Kishiyama and S. Morris, 4/13/94; S.Morris and J. Gee, 4/8/96).

50476-059; 150291: EPA' s evaluation of DPR doc. # 50476-028, rec. # 088943 - Supplementary.

50476-026; 075635: This document contains an exact duplicate of the study at DPR doc. # 50476-028, rec. # 088943.

50476-051; 145631;
50476-052; 145635;
50476-053; 145636;
50476-058; 145649: The registrant submitted: comments on DPR's evaluations; a rationale for not using the registered technical active ingredient; a protocol for a chronic toxicity study; a rationale for the doses used; individual and summary data for clinical signs; rationale for not submitting hematology data; comments on serum chemistry, urinalysis, and ophthalmology data; individual necropsy, pathology, and histopathology data; and a rationale for not submitting histopathology data for the spinal cord. Evaluation of this submission resulted in a change in study status to not upgradeable. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-020; 064202; "Induction of Rat Mammary Adenomas with the Respiratory Inhibitory Rotenone." (Published in Cancer Research 33: 3047 - 3050 (1973)) Rotenone (no purity stated), given by i.p. injection to 4 series of 10 female rats at 1.7 ug/g body weight for 42 days in sunflower oil, 10 vehicle controls; historical incidence of mammary tumors was 0.5/1000 rats/year; in the first series, the incidence of mammary tumors was 100% appearing 6 to 11 months after termination of treatment with none in controls at 19 months; in the other 3 series, the incidence was 60% at 10 months; 8 tumors from the first series were extirpated for morphological studies and transplantation - 7 were adenomas and 1 a differentiated adenocarcinoma; 4 to 5 of 30 tries successful in transplanting tumors; no changes were noted in the liver; UNACCEPTABLE (summary, insufficient information.) (J. Gee, 12/4/87).

50476-021; 064222; "Failure of Rotenone to Interfere with 17 - beta-Estradiol Action in the Rat Uterus." (Published in Cancer Research 39: 4438 - 4440 (1979), Olson and Sheehan) Rotenone (no purity stated); tested in vitro and in vivo for estradiol binding in an effort to define the role in rat mammary carcinogenesis; for in vivo study, groups of 4, ovariectomized rats were implanted with capsules containing combinations of estradiol and rotenone (0, 0.5, 5.0 or 50.0 mg) and the uterus removed and weighed after 5 days; both in vitro and in vivo portions demonstrated that rotenone did not act as an estrogen or estrogen antagonist; Supplementary (summary data for possible mechanism.) (J. Gee, 12/7/87.).

50476-025; 064240; "Minireview: Carcinogenesis with the Insecticide Rotenone." (Published in Life Sciences 32: 809 - 816 (1983), Gosalvez) Rotenone; review of literature on carcinogenesis of rotenone in several species. The author concludes that rotenone is carcinogenic in the rat after oral administration and is enhanced when the diet is deficient, especially in riboflavin. The effective oral dose is stated as 2 to 25 ppm continuously. The incidence of tumors depended on strain of rat, diet and other factors. Recent negative reports from U. S. agencies used enriched diets. Further studies are needed. UNACCEPTABLE (summary). Author identifies rotenone as a possible carcinogen. (J. Gee, 12/16/87).

50476-021; 064212; "Spectral and Metabolic Characteristics of Mitochondrial Fractions from Rotenone-Induced Tumours." (Published in Cancer 36: 243 - 253 (1977), Gosalvez et al.) Rotenone (no purity stated) given by i.p. injection at 1.7 ug/g body weight for 40 to 50 days to Wistar rats or by oral intubation to 9 rats for 45 days at 0.2 mg/rat followed by 15 days at 0.3 mg; mammary fibroadenomas appeared 7 to 10 months after end of i.p. injections, tumors also appeared following oral gavage with no tumors in the controls at 19 months; tumors stated to be histologically benign but could be transplanted; mitochondria from rotenone-induced tumors lacked respiratory control, oxidative phosphorylation and were insensitive to cyanide; UNACCEPTABLE (summary); demonstrates that rotenone induced mammary fibroadenomas. (J. Gee, 12/7/87).

ONCOGENICITY, MOUSE

50476-028; 088943; "Toxicology and Carcinogenicity Studies of Rotenone in F344/N Rats and B63F1 Mice", NIH Pub. No. 88-2576; K. M. Abdo; National Toxicology Program, Battelle Columbus Laboratory; January 1988. Rotenone (lot no. 735-RAP-1502) was given in the feed to 50 B6C3F1 mice/sex/dose for 103 weeks at 0, 600, or 1200 ppm. The only treatment-related effect was a decrease in group mean body weights, relative to controls, in both sexes at 600 and 1200 ppm (non-oncogenic NOEL < 600 ppm). No adverse effect was indicated. The study was unacceptable and not upgradeable with because the registered technical active ingredient was not used and there were no hematology data and no histopathology data for the spinal cord (J. Kishiyama and S. Morris, 4/14/94; S.Morris and J. Gee, 4/8/96).

50476-059; 150212: EPA' s evaluation of NIH Publication No. 86-2576 that appears to be the same study as DPR doc. # 50476-028, rec. # 088943 - Supplementary.

50476-026; 075635: This document contains an exact duplicate of the study at DPR doc. # 50476-028, rec. # 088943.

50476-051;
50476-054; 145637;
50476-054; 145641;
50476-055; 145644;

50476-058; 145649: The registrant submitted: comments on DPR's evaluations; a rationale for not using the registered technical active ingredient; a protocol for a chronic toxicity study; a rationale for the doses used; individual and summary data for clinical signs; rationale for not submitting hematology data; comments on serum chemistry, urinalysis, and ophthalmology data; individual necropsy, pathology, and histopathology data; and a rationale for not submitting histopathology data for the spinal cord. Evaluation of this submission resulted in a change in study status to not upgradeable. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-007; 032712; "Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note." (Innes, et al. J. Nat. Cancer Inst. 42:1101 (1969)) Rotenone, no purity stated, was given by gavage from 7 days old until weaning, then in feed at 0 or 1.0 mg/kg for 18 months to mice, two F_1 hybrid strains, 18/strain/sex/treatment. No adverse effect indicated**.** UNACCEPTABLE**.** Not a guideline type protocol, only 1 dose level, questionable if dosage was anywhere near an ADI [compare to 1000 ppm successfully used in hamsters in 007:32709]. J. Gee, 8-2-85.

50476-020; 064197: This document contains an exact duplicate of the study at DPR doc. # 50476-007, rec. # 088943.

ONCOGENICITY, HAMSTER

50476-007; 032709; "Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters." (Study location not indicated: Project Officer was R. L. Baron of EPA Environ. Toxicology Div., Health Effects Research Lab: April, 1981). Rotenone, 95% purity, was given to 50/sex/group Syrian Golden hamsters at dietary levels of 0, 125, 250, 500 or 1000 ppm for 18 months. Possible adverse effect (adrenal cortical carcinomas seen in one male and 2 females at 1000 ppm, but not in other groups). UNACCEPTABLE, not upgradeable. High mortality possibly due to pathogenic E. coli, no analysis of dose, no individual data. (J. Gee, 8-2-85).

Note: Information which may help DPR to evaluate these results is requested. Such information includes appropriate historical control data on the same strain of hamster obtained from the same source and evaluated in the test facility during a comparable testing period. Individual survival data and time to tumor data for adrenal cortical carcinomas are also requested.

REPRODUCTION, RAT

50476-024; 064229; "A Reproduction Study for Safety Evaluation of Rotenone using Rats", Study No. 81077; K.M. MacKenzie; Hazleton Raltech, Inc., Madison, WI; 2/11/83. Groups of 15 male and 25 female CD(SD)BR rats of the F0 generation were fed rotenone (97-98% purity) in the diet at 0, 7.5, 37.5, or 75 ppm continuously from 6 weeks of age through breeding, gestation, lactation, and weaning of the F1a litters. Selected groups of 15 males and 25 females of the F1a generation were exposed in utero, via mother's milk until weaning, and in the diet through breeding, gestation, lactation, and weaning of the F2a litters at 0, 7.5, 37.5, or 75 ppm. Adult body weight gain was decreased for both sexes in both generations at 37.5 and 75 ppm. A **possible adverse effect** was indicated by decreased mean live litter sizes in the F0 and F1 generations at 75 ppm, decreased mean birth weights of the F1 and F2 pups at 75 ppm and decreased body weight gain of the F1 and F2 pups at 37.5 and 75 ppm. The study is unacceptable and not upgradeable because the registered technical active ingredient was not used (J. Gee, 12/8/87; J. Kishiyama and S. Morris, 8/3/94; S.Morris and J. Gee, 4/8/96).

50476-059; 150213: EPA' s evaluation of DPR doc. # 50476-024, rec. # 064229 - Minimum.

50476-032; 095266: This document contained additional data for the study at DPR doc. # 50476-024, rec. # 064229. Evalution of these data resulted in no change in study status. A worksheet was done (J. Kishiyama and S. Morris, 8/3/94S; Morris and J. Gee, 4/8/96).

50476-051;
50476-057; 145646: The registrant submitted a certificate of purity for the test material (97%, batch no. 100287 and a rationale for not using the registered technical active ingredient. Evaluation of this submission resulted in a change in study status to not upgradeable. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

REPRODUCTION, HAMSTER

50476-007; 032708; "Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters." (EPA, 4-81) Rotenone, 95%, was administered to Syrian Golden hamsters at dietary levels of 0, 500 or 1000 ppm for three months. This is a summary of a one generation study in which the animals were mated twice. Possible adverse effects: smaller testicles and infertility at 1000 ppm, poor litter survival, maternal deaths and cannibalism at 500 ppm and above. UNACCEPTABLE, not upgradeable. Not a guideline type study, no NOEL was established, no data. (J. Gee, 8-2-85).

TERATOLOGY, RAT

50476-025; 064243; "Teratology Study with Rotenone in Rats", Study No. 81178; K.M. MacKenzie; Hazleton Raltech, Inc., Madison, WI; 7/17/82. Rotenone (97-98% stated purity, corn oil vehicle) was given by oral gavage to 25 pregnant female COBS* CD* albino rats/dose on gestation days 6 through 19 at 0, 0.75, 1.5, 3, or 6 mg/kg/day. A treatment-related decrease was seen in body weight gain at 1.5, 3.0, and 6.0 mg/kg/day and increased rubbing of the face and paws on the cage bottom, salivation prior and after treatment, lethargy, reddish-brown tinge on fur, reddish brown nasal exudate, and rough coat were seen at 0.75, 1.5, 3.0, and 6.0 mg/kg/day (maternal NOEL < 0.75 mg/kg/day). Decreased fetal weight and increased incidence of unossification of the fifth and sixth fetal sternebrae were seen at 6 mg/kg/day. The developmental NOEL was set at 1.5 mg/kg/day because of uncertainties about the analysis of the 3 mg/kg/day dosing material. A **possible adverse effect** was indicated by developmental effects being present at a dose that showed mild maternal effects. The study was unacceptable and not upgradeable because the registered technical active ingredient was not used (J. Gee, 12/16/87; J. Kishiyama and S. Morris, 4/22/94; S.Morris and J. Gee, 4/8/96).

50476-059; 150216: EPA' s evaluation of DPR doc. # 50476-025, rec. # 064243 - Minimum.

50476-030; 092534: This document contains additional data for the study at DPR doc. # 50476-025, rec. # 064243. Evaluation of these data indicated a possible adverse effect (J. Kishiyama and S. Morris, 4/22/94).

50476-051: The registrant submitted a rationale for not using the registered technical active ingredient. Evaluation of this submission resulted in a change in study status to not upgradeable. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-025; 064244; "Teratogenicity Study on Pyrethrum and Rotenone (Natural Origin) and Ronnel in

Pregnant Rats." (Published in J. Toxicol. Environ. Health 10: 111-119 (1982), Khera et al.) Rotenone technical, 87% and 13% other cube extractives (S. B. Penick, lot 350-LAO); 20 Wistar rats/group, given by oral gavage, days 6 - 15 (day of positive vaginal smear = day 1) at 0, 2.5, 5.0 or 10.0 mg/kg (not corrected for purity) at 10 ml/kg; 2/3 for skeletal, 1/3 for visceral exam; 12/20 died at 10 mg/kg, body weight gain reduced at 5 and 10 mg/kg; increase in resorptions at 10 mg/kg; developmental NOEL = 2.5 (lower fetal weight, skeletal anomalies), maternal NOEL = 2.5 mg/kg (decreased body weight gain, mortality); UNACCEPTABLE (no individual data, summary). No effect on the fetus without maternal effect(s). (J. Gee, 12/17/87).

50476-024; 064230; "Reproductive Responses to Rotenone During Decidualized Pseudogestation and Gestation in Rats." (Published in Bull. Environ. Contam. Toxicol. 28: 360 - 368 (1982), Spencer and Tat Sing.) Rotenone (no purity stated) fed in the diet to decidualized pseudopregnant rats, 10/group, at 0, 10, 100, 200, 250, 500, 750 or 1000 ppm, days 6 to 10 and to pregnant rats, 7/group, at 0, 10, 100, 200, 400, 600 or 800 ppm, days 6 - 15; Sprague-Dawley rats; protein and glycogen content of the uterus (pseudopregnant rats) and ovaries and placenta (pregnant rats) was determined; apparent NOEL (pseudopregnant rats) = 500 ppm (body weight and clinical signs of lethargy and ataxia); maternal NOEL = 200 (body weight loss, clinical signs); developmental NOEL = 10 ppm (decreased "fetal survival per litter at birth"; Supplementary data - study was not designed as a teratology study. Possible adverse effect on fetuses between day 12 and birth. (J. Gee, 12/8/87).

Summary: Possible adverse effects were seen in the studies at doc. #'s 064243 and 064230 but not in the study at doc. # 064244. This may be due to differences in the strain of rat, route of administration (diet versus gavage) or dose levels (800 ppm in doc. # 064230 is roughly equivalent to 28 mg/kg/day). The study at doc. # 064244 is not upgradeable because there were too few live litters (14) in the high dose group. The study at doc. # 064230 did not follow a teratology protocol and is therefore classified as supplemental data. The status for the rat teratology test type is based on the findings in the study at doc. # 064243 which is unacceptable and not upgradeable because the registered technical active ingredient was not used (S.Morris, 4/8/96).

TERATOLOGY, RABBIT

No study on file.

TERATOLOGY, MOUSE

50476-025; 064242; "Teratology Study with Rotenone in Mice", Raltech Study No. 80050; K.M. MacKenzie; Raltech Scientific Services, Madison, WI; 11/24/81. Rotenone (98.2% stated purity, corn oil vehicle) was given by oral gavage to 30 pregnant female CD-1 mice/dose on gestation days 6 through 17 at 0, 3, 9, or 15 mg/kg/day. On gestation day 18 the mice were sacrificed. Their reproductive tracts were removed, weighed, and examined. All fetuses were sexed, weighed, and examined. One-half of the fetuses were freehand dissected and examined for visceral abnormalities. All whole and dissected fetuses were cleared and stained and examined for skeletal malformations. There were no treatment-related maternal or developmental effects. A **possible adverse effect** was indicated by decreased live litter size and increased fetal resorption seen in a pilot study (DPR doc. # 50476-057, rec. # 145646) at a dose, 24 mg/kg/day, that does not produce frank maternal toxicity (maternal NOEL >= 24 mg/kg/day, developmental NOEL = 12 mg/kg/day). The study was unacceptable and not upgradeable because the registered active ingredient was not used, inadequate analytical data, and inadequate rationale for the doses used (J. Gee, 12/16/87; J. Kishiyama and S. Morris, 5/5/94; S. Morris and J. Gee, 4/8/96).

50476-059; 150217: EPA' s evaluation of DPR doc. # 50476-025, rec. # 064242 - Minimum.

50476-031; 095265: This document contained additional data for the study at DPR doc. # 50476-025, rec. # 064242. Evaluation of these data did not result in a change in study status. A worksheet was done (J. Kishiyama and S. Morris, 5/5/94).

50476-025; 064241: This document contains a dose range-finding study for the study at DPR doc. # 50476-025, rec. # 064242. These data were evaluated on the worksheet for DPR doc. # 50476-031, rec. # 095265. Evaluation of these data did not result in a change in study status (J. Kishiyama and S. Morris, 5/5/94).

50476-051;
50476-057; 145646: The registrant submitted a rationale for not using the registered technical active ingredient, no analytical data, a complete copy of Raltech Study No. 80049, and a rationale for the doses used. Evaluation of this submission resulted in a change in study status to not upgradeable and the finding of a possible adverse effect. See DPR Worksheet 4/8/96 (S.Morris and J. Gee, 4/8/96).

GENE MUTATION

50476-033; 095363; "The Salmonella/Microsome Mutagenicity Test System (Rotenone)"; SRI International; no date. Rotenone (purity not stated, DMSO or ethanol vehicle) was tested at 0, 100, 333, 1000, 3333, or 10000 mg/plate with or without metabolic activation system (S9 fraction of Aroclor 1254-induced male Syrian hamster or Sprague Dawley rat liver homogenates) for the induction of prototrophic mutants from histidine auxotrophic bacterium using Salmonella typhimurium tester strains TA98, TA100, TA1535, and TA1537. Precipitation was seen at 1000, 3333, and 10000 mg/plate. A treatment-related increase in revertants was not seen. No adverse effect was indicated. The study was unacceptable but possibly upgradeable with adequate submissions of analysis of the test article and dosing material, cell survival data, individual data, study dates, and GLP and QA sign-off sheets and a detailed protocol of the cell culture techniques, preparation and use of the activation system, exposure conditions, and concentrations of the positive controls (J. Kishiyama and S. Morris, 5/17/94).

50476-059; 150219: EPA' s evaluation a report that appears to be the same study as DPR doc. # 50476-033, rec. # 095363 - Acceptable.

50476-028; 088943: Page 138 of this document contains a summary table of some of the data from the study at DPR doc. # 50476-033, rec. # 095363.

50476-033; 095358: This document contains a partial duplicate of the study at DPR doc. # 50476-028, rec. # 088943.

50476-033; 095366; "Mouse Lymphoma Protocol"; Inveresk Research International; 4/24/87. Rotenone (acetone vehicle) was tested in duplicate at 0, 0.5, 1.0, 2.0, 4.0, or 8.0 in trial 1 and 0, 0.25, 0.5, 1.0, 2.0, or 4.0 mg/ml in trial 2. Mouse L5178Y lymphoma cells were exposed for 4 hours, washed, grown up for 2 days, and then subcultured in the presence of trifluorothymidine for 10 - 12 days. A **possible adverse effect** was indicated by a treatment-related increase in trifluorothymidine-resistant colonies. The study was unacceptable and not upgradeable because of inadequate analytical data for the test article and dosing materials, no cell culture methods, incomplete protocol, illegible copies of the individual data and no trials with metabolic activation (J. Kishiyama and S. Morris, 5/20/94).

50476-059; 150220: EPA' s evaluation a report that appears to be the same study as DPR

doc. # 50476-033, rec. # 095366 - Acceptable.

50476-028; 088943: Page 139 of this document contains a summary table of the data from the study at DPR doc. # 50476-033, rec. # 095366.

50476-033; 095358: This report contains a summary table of the data from the study at DPR doc. # 50476-033, rec. # 095366.

50476-033; 095360; "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenesis Assay (Rotenone)", Study # 019-563-165-1; S.R. Haworth; EG & G Mason Research Institute, Rockville, MD; 11/3/78. Rotenone (unstated purity, DMSO vehicle) was tested for its effect on the mutation rate of histidine auxotrophic strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1538) to prototrophy. Two trials were conducted with 3 plates/dose/strain at 0, 30, 100, 330, 1000, 3300, or 10000 mg/plate with or with without metabolic activation (S9 fraction of Aroclor 1254 induced male Sprague-Dawley rat liver homogenates). There were no treatment-related effects on mutation rates. No adverse effect was indicated. The study was unacceptable and not upgradeable because the registered technical active ingredient was not used and there were no positive controls with metabolic activation for tester strains TA1535, TA1537, and TA1538 and the no data for the first TA1537 trial (J. Kishiyama and S. Morris, 8/4/94; S.Morris and J. Gee, 4/8/96).

50476-059; 150218: EPA' s evaluation of DPR doc. # 50476-033, rec. # 095360 - Acceptable.

50476-051; rec. # 145631:The registrant submitted comments on DPR's evaluations, a rationale for not using the registered technical active ingredient, the nominal purity of the test material and no data. Evaluation of this submission resulted in a change in study status to not upgradeable. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-007; 032702; "Mutagenic Activity of Several Pesticides (Rotenone) Using the Salmonella Test and Saccharomyces D3 System - Salmonella/Mammalian Microsome Mutagenicity (Ames) Test." (Alabama A & M University, 1978) Abstract stated there was no mutagenic effect observed, but no data was presented. UNACCEPTABLE, not upgradeable. (J. Gee, 8-2-85).

50476-036; 091300: This document summarizes the negative results of rotenone tested in 14 genetic toxicity assays. No adverse effect was indicated. No worksheet was done (S. Morris, 9/8/94).

CHROMOSOME EFFECTS

50476-033; 095358; "Toxicology and Carcinogenesis Studies of Rotenone in F344/N Rats and B63F1 Mice"; K. M. Abdo; National Toxicology Program; Litton Bionetics Inc.; January 1988. Rotenone (acetone vehicle) was tested in a sister chromatid exchange assay in which Chinese Hamster ovary cells were exposed for 2 hours at 0, 0.001, 0.003, 0.004, or 0.008 mg/ml (trial 1) without metabolic activation (-S9) or with metabolic activation (+S9, S9 fraction of Aroclor 1254-induced male Sprague-Dawley rats liver homogenates) at 0, 0.2, 0.6, 2, 6, or 20 mg/ml (trial 1) or 0, 6, 10.1, 15.2, or 20 mg/ml (trial 2). Following exposure, BrdU was added directly to the -S9 cells while the +S9 were washed first. The -S9 cells were incubated 34.5 hours and the +S9 cells 34.0 (trial 1) or 35.5 (trial 2) hours. Colcemid was present in the final 2-3 hours of incubation. Cells were then collected by mitotic shake-off, fixed, air-dried, stained and scored for sister chromatid exchanges (SCEs). A **possible adverse effect** was indicated by a treatment-related increase in SCEs with S9 in trial 1. The study was unacceptable and not upgradeable because only one trial with a single culture was done without S9.

The equivocal nature of the possible adverse effect might be resolved by adequate submissions of a complete copy of the study that includes: the protocol used, analysis of the test article and dosing material, cell survival data, cell cycle data, cell culture methods, rationale for the treatment levels, data and rationale supporting selection of the exposure conditions, all individual data, and documentation of good laboratory practice and quality assurance (J. Kishiyama and S. Morris, 5/9/94; S. Moris and J. Gee, 4/8/96).

50476-051;
50476-057; 145648: The registrant submitted comments on DPR's evaluations, protocols for the study, and no data. Evaluation of this submission resulted no change in the "not upgradeable" study status. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-033; 095358; "Toxicology and Carcinogenesis Studies of Rotenone in F344/N Rats and B63F1 Mice"; K. M. Abdo; National Toxicology Program; Litton Bionetics Inc.; January 1988. Rotenone (acetone vehicle) was tested in a chromosome aberration assay in which Chinese hamster ovary cells were exposed for 8 - 10 hours without metabolic activation system (-S9) at 0, 10, 25, or 50 mg/ml (trial 1) and 0, 25, 75, or 100 mg/ml (trial 2) or 2 hours with metabolic activation (+S9, S9 fraction of Aroclor 1254-induced male Sprague-Dawley rat liver homogenates) at 0, 100, 150, 200, or 250 mg/ml (trial 1). The cells were washed, fresh medium was added, and incucbation continued for a total of 21.5 (trial 1) or 20.5 (trial 2) hours. Colcemid was added for the last 2 - 3 hours of incubation. Cells were harvested by mitotic shake-off, fixed, stained, and scored for chromosome aberrations. There were no treatment-related increase in chromosome aberrations. No adverse effect was indicated. The study was unacceptable and not upgradeable because only one trial was done without S9 and the report was missing: the protocol used, analysis of the test article and dosing material, cell survival data, cell cycle data, cell culture methods, rationale for the treatment levels, data and rationale supporting selection of the exposure conditions, all individual data, and documentation of good laboratory practice and quality assurance (J. Kishiyama and S. Morris, 5/11/94).

50476-051;
50476-057; 145648: The registrant submitted comments on DPR's evaluations, protocols for the study, and no data. Evaluation of this submission resulted no change in the "not upgradeable" study status. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-033; 095359; "In Vitro and In Vivo Mutagenicity Studies of Environmental Chemicals (includes Rotenone)", Report No. EPA-600-1-84-003; D.C.L. Jones, et al.; SRI International; 9/84. Rotenone (41.59% stated purity, DMSO vehicle) was given by an unspecified route to groups of male Swiss-Webster mice at 0, 0.56. 1.13, or 2.25 mg/kg on hours 0 and 24. Eight mice/dose were sacrificed at 48, 72, or 96 hours. Two cardiac blood and 3 bone marrow smears/mouse were taken at sacrifice. After staining and drying, 500 polychromatic erythrocytes (PCE's)/mouse were examined for micronuclei and the ratio of PCE's/mature red cells (RBC's) was determined. There were no treatment-related effects on micronuclei. No adverse effect was indicated. The study was unacceptable and not upgradeable because there were no analytical data for the test material or the dosing material, only males were used, there were no data supporting the selection of doses, the dosing route was not stated, insufficient PCE's and RBC's were scored, illegible tabulated data, and unclear sacrifice times (J. Kishiyama and S. Morris, 8/10/94).

50476-024; 064232; "Mutagenicity Studies on Rotenone: Final Report", LBI Project No. 22063; D. Brusick; Litton Bionetics, Inc., Kensington, MD; 6/24/81. Rotenone (97% stated purity, ethanol vehicle) was tested for induction of reverse mutations methionine auxotrophic strains of Saccharomyce cerevisiae (S138, S211) to prototrophy. One plate/trial was exposed to 0, 1, 10, 100, 500, 1000, 2500, 5000, or 10000 mg/plate with metabolic activation (S9 fraction of Aroclor 1254-induced male Sprague-

Dawley rat liver homogenates; S211 - 1 trial, S138 - 3 trials) or without activation (S211, S138 one trial each). There was no treatment-related increase in revertant colonies. No adverse effect was indicated. The study was unacceptable and not upgradeable because the technical active ingredient was not used, the analytical data were inadequate, only one replicate was used, cytotoxicity data were not presented, the S9 mixture was not adequately characterized, the positive controls were inadequate, and there was no statistical analysis (J. Gee, 12/9/87; J. Kishiyama and S. Morris, 8/17/94).

50476-033; 095364: This document contains additional data for the study at DPR doc. # 50476-024, rec. # 064232. Evaluation of these data resulted in no change in study status. A worksheet was done (J. Kishiyama and S. Morris, 8/17/94).

50476-024; 064231; "Analytical Studies for the Detection of Chromosomal Aberrations in Fruit Flies, Rats, Mice, and Horse Bean." (Biotech Research Laboratories, Inc., MD, 7/20/81, for the U. S. Fish and Wildlife Service) Rotenone, 98%, given by oral gavage to 10 rats (sex distribution not stated) at three doses with the high dose being 1/10 the LD_{50} (71 mg/kg) as determined in a toxicity test in 20 rats/dose at 50, 100, 200 and 300 mg/kg body weight; incomplete report with all data missing; UNACCEPTABLE (incomplete and high dose probably inadequate as no toxicity is reported in the text). No increase in aberrations or change in mitotic index reported. Not upgradeable. (J. Gee, 12/9/87).

EPA 1-liner: Unacceptable.

50476-051;
50476-057; 145648: The registrant submitted comments on DPR's evaluations, protocols for the study, and no data. Evaluation of this submission resulted no change in the "not upgradeable" study status. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-024; 064231; "Analytical Studies for the Detection of Chromosomal Aberrations in Fruit Flies, Rats, Mice, and Horse Bean." (Biotech Research Laboratories, MD, 7/20/81, for U. S. Fish and Wildlife Service.) Mouse micronucleus test. Rotenone, 98%, given by oral gavage to 8/group (sex distribution not stated), at two doses with the high dose at 1/8 the LD_{50} but doses are not given; report is incomplete with all data missing but text states no treatment-related effect was observed for micronuclei formation; UNACCEPTABLE (no data, high dose probably inadequate since no comments on toxicity are made.) Not upgradeable. (J. Gee, 12/9/87).

EPA 1-liner: Unacceptable.

50476-051;
50476-057; 145648: The registrant submitted comments on DPR's evaluations, protocols for the study, and no data. Evaluation of this submission resulted no change in the "not upgradeable" study status. See DPR Response 4/8/96 (S.Morris and J. Gee, 4/8/96).

50476-036; 091300: This document summarizes the negative results of rotenone tested in 14 genetic toxicity assays. No adverse effect was indicated. No worksheet was done (S. Morris, 9/8/94).

DNA DAMAGE

50476-024; 064232; "Mutagenicity Studies on Rotenone: Final Report", LBI Project No. 22063; D. Brusick; Litton Bionetics, Inc., Kensington, MD; 6/24/81. Rotenone (97% stated purity, ethanol vehicle) was tested for induction of reverse mutations methionine auxotrophic strains of Saccharomyces cerevisiae (S138, S211) to prototrophy. One plate/trial was exposed to 0, 1, 10, 100, 500, 1000, 2500, 5000, or 10000 mg/plate with metabolic activation (S9 fraction of Aroclor 1254-induced male Sprague-

Dawley rat liver homogenates; S211 - 1 trial, S138 - 3 trials) or without activation (S211, S138 one trial each). There was no treatment-related increase in revertant colonies. No adverse effect was indicated. The study was unacceptable and not upgradeable because the technical active ingredient was not used, the analytical data were inadequate, only one replicate was used, cytotoxicity data were not presented, the S9 mixture was not adequately characterized, the positive controls were inadequate, and there was no statistical analysis (J. Gee, 12/9/87; J. Kishiyama and S. Morris, 8/17/94).

> 50476-033; 095364: This document contains additional data for the study at DPR doc. # 50476-024, rec. # 064232. A worksheet was done (J. Kishiyama and S. Morris, 8/22/94).

50476-024; 064232; "Mutagenicity Studies on Rotenone, Final Report", LBI Project No. 22063; D. Brusick; Litton Bionetics, Inc., Kensington, MD; 6/24/81. Rotenone (97% stated purity, ethanol vehicle) was tested for mitotic recombination in Saccharomyces cerevisiae strain D5 that converts heterozygous alleles for pigmentation to homozygous. One trial without and two trials with metabolic activation system (S9 supernatant from Aroclor 1254-induced, male Sprague-Dawley rat liver homogenates) were conducted at 0, 1, 10, 100, 1000, or 10000 mg/ml. Greater than 38800 colonies/dose were scored for cross-over events (color changes). There was no treatment-related increase in cross-over events. No adverse effect was indicated. The study was unacceptable but possibly upgradeable with adequate submissions of characterization of the test material, a rationale for not using the registered technical active ingredient, the number of replicates, and rationale for the doses (J. Gee, 12/9/87; J. Kishiyama and S. Morris, 8/22/94).

> 50476-033; 095364: This document contains additional data for the study at DPR doc. # 50476-024, rec. # 064232. A worksheet was done (J. Kishiyama and S. Morris, 8/22/94).

50476-020; 063484; "Neoplasm Studies. XI. The Effects in Tissue Culture of N,N,N',N'-Tetramethyl-o-Phenylenediamine and Other Compounds on Malignant Lymph Nodes." Published in Cancer Research 3: 293 - 295 (1943) Rotenone was one of several compounds tested in viability experiments at 0.2 and 0.1 "saturation" with lymphocytes from a variety of lymphoid tissues such as lymph nodes, lymphosarcoma, lymphatic leukemia and Hodgkin's disease. The toxic effect of rotenone was nonspecific and was evident only after 48 to 72 hours. UNACCEPTABLE (summary only), no adverse effect identified. (J. Gee, 12/3/87).

50476-020; 063486; "The Cytocidal Action of Mitotic Poisons on Lymphocytes in vitro." (Medical Research Council Radiobiological Unit, Harwell, UK. Published in Biochemical Pharmacology 5: 53 - 63 (1960), O. A. Trowell.) Rotenone, 99%, in water/ethanol; tested with lymph node organ cultures from rats with more than 99% of lymphocytes as mature small lymphocytes; percent dead lymphocytes measured at 48 hours at 3×10^{-8}, 10^{-8}, 3×10^{-9}, 10^{-9} and 3×10^{-10}; LC was 2.7×10^{-9} for lymphocytes and was the most effective "poison" of the compounds tested. UNACCEPTABLE (summary, protocol). The study did not identify genotoxic effects other than lethality. (J. Gee, 12/3/87).

50476-021; 064207; "A New Culture Model Facilitating Rapid Quantitation Testing of Mitotic Spindle Inhibition in Mammalian Cells." (Published in J. Nat. Cancer Inst. 56: 357 - 363 (1976), Brabander et al.) Rotenone, no purity stated; one of a series of compounds tested in vitro, tested with MO cells (epithelioid-type C3H mouse embryo cell line showing contact inhibition) at 1, 10 or 100 ug/ml for 48 hours, then scored for multinucleation; rotenone was "toxic" at 100 ug/ml and induced multinucleation at the two lower concentrations - no data - causing effects "identical" to those of colchicine including a total absence of microtubules. UNACCEPTABLE (summary). Possible adverse effect on mitosis. (J. Gee, 12/7/87).

50476-024; 064231; "Analytical Studies for the Detection of Chromosomal Aberrations in Fruit Flies, Rats, Mice, and Horse Bean." (Biotech Research Laboratories, MD, 7/20/81, for U. S. Fish and Wildlife

Service) Rotenone, 98%; fed to male flies (Drosophila melanogaster, F125, B/Y+) on sucrose-agar medium for 24 hours, 25 flies per group; preliminary toxicity test at 0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 mM; for the full study, the high concentration was 1/10 the LD_{50} (3.4 mM); mated with virgin females (d 63 inscy) in single pairs for 24 hours; newly hatched adults were scored for sex chromosome loss characterized by males with normal eyes and yellow bodies; data corrected for non-disjunction from scoring females with bar eyes and apricot bodies; fewer than half of mating pairs produced offspring; UNACCEPTABLE (all tables of data are missing.) Report states the results were "inconclusive". Full study with tables should be submitted. (J. Gee, 12/9/87).

EPA 1-liner: Unacceptable.

50476-024; 064233; "Mutagenicity Studies on Rotenone." (Midwest Research Institute, Project No. 7029-E, 4/1/81) Rotenone, 99%, was tested for genotoxicity with two strains of Escherichia coli, W3110 (pol A^+) and p3478 (pol A^-) with and without activation in the spot test and in a liquid suspension. In the spot test, the test material was added in 10 ul to a 6.35mm disk at 0, 150, 750 or 1500 ug/plate in duplicate with bacteria incorporated in the top agar. In liquid suspension, bacteria were incubated for 90 minutes at room temperature in triplicate, then plated. Tested at 0, 0.1, 1, 5, 10 or 50 ug/ml - precipitation at 50 ug/ml. Rotenone was stated as not diffusing in the spot test = no test. No differential survival was noted in the liquid incubation test. No adverse effect. (J. Gee, 12/10/87). Study status was changed to unacceptable and not upgradeable because the registered technical active ingredient was not used (S. Morris and J. Gee, 4/8/96).

50476-033; 095365: This document contains an exact duplicate of the study at DPR doc. # 50476-024, rec. # 064233.

50476-024; 064234; "Pesticide Induced DNA Damage and Its Repair in Cultured Human Cells." (The Ohio State University, published in Mutation Research 42: 161 - 174 (1977), F. E. Ahmed, et al.) Rotenone, no purity stated, one of 13 pesticides tested; SV-40 transformed human fibroblast line VA-4, with and without rat liver activation, at 0, 1, 10 and 1000 uM; data presented for 8 hour exposure; hydroxyurea to suppress semiconservative DNA replication; unscheduled DNA synthesis by [^{3}H]-thymidine incorporation and autoradiography; data for rotenone presented as "-" with and without activation; UNACCEPTABLE (summary only with no data). (J. Gee, 12/10/87).

50476-059; 150221: EPA' s evaluation of DPR doc. # 50476-024, rec. # 064234 - Unacceptable

50476-033; 095361: This document contains an exact duplicate of the study at DPR doc. # 50476-024, rec. # 064234.

50476-024; 064236; "Action of Rotenone and Related Respiratory Inhibitors on Mammalian Cell Division. 1. Cell Kinetics and Biochemical Aspects." (Published in Cytobios 15: 85 - 96 (1976), Barham and Brinkley) Rotenone (no purity stated), tested with CHO cells at 1.2 x 10^{-5} for the effect on several parameters of cell cycle progression. CHO were synchronized at G_1-S by excess thymidine, at mitosis by mitotic shake-off. Asynchronous populations were also used. Study concluded that rotenone inhibited the mitotic process by direct interference with spindle microtubule assembly. Cell cycle progression was delayed possibly associated with rotenone's affect on respiration and energy production. UNACCEPTABLE (summary), possible adverse effect on mitosis and cell cycle progression. (J. Gee, 12/14/87).

50476-024; 064236; "Action of Rotenone and Related Respiratory Inhibitors on Mammalian Cell Division. 2. Ultrastructural Studies." (Published in Cytobios 15: 97 - 109 (1976), Barham and Brinkley) Rotenone (no purity stated); tested with CHO cells at 1.2 x 10^{-5} M; scanning and transmission electron

microscopy of cells exposed for 3 hours; microscopy revealed that cells did not progress beyond early stages of mitosis but progressed after removal of rotenone; interfered with microtubule assembly; UNACCEPTABLE (summary), possible adverse effect on mitosis. (J. Gee, 12/15/87).

50476-024; 064237; "Metaphase Arrest of Chinese Hamster Cells with Rotenone." (Published in Experimental Cell Res. 42: 291-295 (1966), Meisner and Sorensen) Rotenone (S. B. Penick) added at between 15 and 150 ul (1×10^{-7} to 1×10^{-5} M) to the Don strain of Chinese hamster cells for 15 minutes to 8 hours; measured the mitotic index of 4000 cells at each concentration and time (hours); increase in mitotic index which was not reversed by adding succinate to bypass block in respiration; UNACCEPTABLE (summary), possible adverse effect on mitosis. (J. Gee, 12/15/87).

50476-036; 091300: This document summarizes the negative results of rotenone tested in 14 genetic toxicity assays. No adverse effect was indicated. No worksheet was done (S. Morris, 9/8/94).

50476-036; 091299: This document contains a paper from the open literature that reports negative results for rotenone in the mouse bone marrow micronuclei assay. No adverse effect was indicated. No worksheet was done (S. Morris, 9/8/94).

Summary: The effect of rotenone identified in several studies is on microtubule assembly with interference on the progression through mitosis. There were negative findings in several tests with other endpoints such as unscheduled DNA synthesis and differential growth of bacteria. Altough a possible adverse effect is indicated the data gap is not filled because these studies were performed either with purified rotenone or an uncharacterized test material and not the registered technical active ingredient (S. Morris, 4/8/96).

NEUROTOXICITY

Not required at this time.

SUPPLEMENTAL DATA

Subchronic, Rat

Note: Studies below were previously reviewed as "oncogenicity" studies, however were not found suitable for chronic or oncogenicity evaluation due to limitations in study design. One-liners are listed here only because DPR reviews are on record (C. Aldous).

50476-007; 032710; "Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters." (Study location not indicated: Project Officer was R. L. Baron of EPA Environ. Toxicology Div., Health Effects Research Lab: April, 1981). Summary article, rotenone, 95% purity, was administered to Wistar rats (25/sex/group) by gavage for 42 consecutive days at 0, 1.7 or 3.0 mg/kg. Rats were then observed for 13 months. No oncogenic effects. UNACCEPTABLE, not upgradeable. No individual data, the 42-day treatment protocol is unacceptable as a chronic or oncogenicity study, only 2 dose levels, no analysis of dose. Original review by J. Gee, 8-2-85, indicated "suggestive evidence" of a possible adverse effect (adrenal cortical adenoma in females at both dose levels). Data was re-examined by C. Aldous and J. R. Gee on (10/26/87), and reviewers concluded that data did not indicate an increase in such tumor incidence.

50476-007; 032711; "Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal

Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters." (EPA, 4-81) Summary article, rotenone, 95%, was given by intraperitoneal administration to Sprague-Dawley rats (25/sex/group) for 42 consecutive days at 0, 1.7 or 3.0 mg/kg. Rats were then observed for 17 months. Incidence of myocardial fibrosis was elevated at both dose levels and in both sexes [but findings were not dose-related]. UNACCEPTABLE. Not a guideline type study, no individual data. (J. Gee, 8-2-87).

Miscellaneous Studies

Note: These studies are included as supplementary data. They do not conform with the SB950 requirements but do indicate positive responses in related systems.

50476-020; 064198; "Teratogenic Effects of Rotenone on the Early Development of Chick Embryos in vitro." (Published in Teratology 4: 191-198 (1971), Rao and Chauhan) Rotenone (no purity stated), tested with chick embyros for 15 minutes at 0.5, 0.8 or 1.0 ug/ml; embryos at 5 developmental stages were used, 10 - 16 per stage; **possible adverse effect** - rotenone arrested development at some stages especially stages 4 and 5; ATP was quite effective in reversing the effects as anticipated by the mechanism of action of rotenone on the mitochondrial respiratory chain. Supplemental data. (J. Gee, 12/4/87).

> 50476-023; 064198: This document contains an exact duplicate of the study at DPR doc. # 50476-023, rec. # 064198.

50476-024; 064235; "DNA Strand Scission and Its Repair Following Exposure of Cells to Inhibitors of Oxidative Phosphorylation." (Published in Biochem. Biophys. Res. Commun. 75: 909 - 914 (1977), Hilton and Walker) Rotenone, no purity stated; tested at 10^{-7} M with mouse leukemia L1210 cells and HeLa cells, prelabeled for 2 - 3 generations with [^{14}C]-thymidine and exposed for 60 minutes at 37^{o} in glucose-free medium; cells were lysed 30 minutes in 0.03M NaOH, neutralized, sonicated and single and double stranded DNA separated on hydroxylapatite. In glucose-free medium, the proportion of double-stranded DNA was decreased from 0.78 to 0.35 and was associated with ATP depletion. The strand breaks were repaired in complete medium which included glucose. Supplemental data. (J. Gee, 12/10/87).

> Note: This study was evaluated as showing that rotenone increases strand breaks in DNA under conditions in which ATP is depleted. Such a result would not have been detected under usual post-treatment incubation conditions in complete medium.

50476-022; 064225; "Status Report on Toxicity and Carcinogenicity of Rotenone." (National Institutes of Health, 1974) Review of the literature pointing out mammary tumors in rats and liver changes in dogs and rats. No worksheet. (J. Gee, 12/7/87).

50476-022; 064226; "Rotenone" Pre-RPAR Review." (EPA Office of Pesticides and Toxic Substances, 5/80 and 6/81, completion of the RPAR review) The oncogenicity studies are reviewed with a discussion of the flaws. The conclusion is that rotenone does not present an oncogenic potential. The data for mutagenicity were considered insufficient and a full battery of tests recommended. Further testing of developmental effects in two species and reproductive effects in rats are also recommended due to flaws in those conducted previously. (J. Gee, 12/7/87).

50476-033; 095362; "Stillwell and Gladding Study on the Relative Safety of Rotenone: Cytotoxicity and Tumor Promoting Activity of Fresh and Degraded Rotenone Resins"; L.J. Maltese and T. Hartman; Stillwell and Gladding Testing Laboratories, New York; NY 7/8/85. Inhibition of metabolic cooperativity between HG-PRT (-) and HG-PRT (+) strains of cultured Chinese hamster lung fibroblast (V-79) cells

was tested using fresh (0, 10, 50, 100, or 250 ng/ml) or degraded (0, 50, 100, 250, 500, or 750 ng/ml) rotenone (purities and lot nos. not stated, DMSO vehicle). The lack of survival of HG-PRT (-) cells cocultured with HG-PRT (+) cells in the presence of the test material and 6-Thioguanine indicated the test material did not inhibit metabolic cooperativity. There were adequate cytotoxic, positive, and negative controls. No adverse effect was indicated. No worksheet was done (S. Morris, 8/3/94).

> 50476-059; 150222: EPA' s evaluation of DPR doc. # 50476-033, rec. # 095362 - Unacceptable

50476-033; 095364; "Mutagenicity Studies on Rotenone: Final Report", LBI Project No. 22063; D. Brusick; Litton Bionetics, Inc., Kensington, MD; 6/24/81. Rotenone (97% stated purity, DMSO or DMSO/corn oil vehicle) was given by oral gavage to groups of 4 to 16 pregnant (of 25 to 91 mated/group) female C57B1/6 mice on gestation days 8, 9, 10 and 11 in DMSO at 0, 0.05, 0.17, 0.5, or 1.0 mg/kg; in DMSO/ corn oil (50%:50%) at 0.05, 0.17, or 1.0 mg/kg; or in corn oil alone at 1000 mg/kg. Pups were scored for coat color spots caused by mutations in coat color genes of melanocyte precursor cells. A treatment-related effect was not seen. No adverse effect was seen. This was not a guideline study type. A worksheet was done (J. Gee, 12/9/87) and the one-liner updated (S. Morris, 8/18/94).

> 50476-024; 064232: This document contains a partial duplicate of the study at DPR doc. # 50476-033, rec. # 095364. The original worksheet used these data (J. Gee, 12/9/87).

50476-060; 151307; "General Metabolism Study for Safety Evaluation of Rotenone using Rats", Project No. 419-137; Hazleton Laboratories America, Inc., Vienna VA; 10/16/84. Male and female Sprague Dawley rats (Charles River Breeding Laboratories, Kingston, NY) were dosed with purified rotenone (Aldrich Chemical Co., Milwaukee, WI, lot #'s 800507, 801110, 99.23% pure). Acute LD_{50}'s were determined by giving 10 rats/sex/group single oral gavages of rotenone (corn oil vehicle, 1 ml/100 grams body weight) at 10, 25, 50, 75, or 150 mg/kg. Preliminary excretion balance, excretion balance, pharmacokinetic, and enterohepatic circulation studies were conducted in both sexes using oral and intravenous (iv) doses of ^{14}C-rotenone at 0.01 to 5.0 mg/kg. Urinary and fecal metabolites were analyzed by thin layer chromatography. Male and female LD_{50}'s were respectively 102 and 39.5 mg/kg. Feces were the major route of excretion for iv and oral exposures with small amounts of ^{14}C being recovered in the urine. Excretion was nearly complete in 48 hours in both sexes after iv doses and in males after oral doses. Female excretion was nearly complete 72 hours after oral dosing. Tissue retention of ^{14}C was low. The pharmacokinetics data were described by a two-compartment model. Data were consistent with enterohepatic recycling. Nine metabolites were detected in urine and feces. Four of these cochromatographed with ^{14}C-labeled impurities in the standard. No parent compound could be detected in feces or urine. The major metabolite (S0) was very polar and in feces accounted for 40.82 to 72.99% of the excreted ^{14}C by males and 33.48% to 65.76% by females. A similar polar metabolite in male urine accounted for 69.67% to 93.37% of the excreted ^{14}C and in females 43.51 to 94.88%. Only one metabolite (S8) from one rat (BO5481) comigrated with a known standard (rotenolone). It represented about 0.23% of the dose (8.2% of the urinary ^{14}C X 2.79% of the dose that was excreted in the urine). It was also an impurity in the ^{14}C-rotenone standard. No worksheet was done. See DPR Response 12/18/96 (S. Morris, 12/18/96).

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